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(71) Applicant *(for all designated States except US)*: CAMBRIDGE SCIENTIFIC, INC. [US/US]; 195 Common Street, Belmont, MA 02178 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CATTANEO, Maurice, V. [CA/US]; 195 Samoset Avenue, Quincy, MA 02169 (US). GRESSER, Joseph, D. [US/US]; 40 Salisbury Road, Brookline, MA 02146 (US). TRANTOLO, Debra, J. [US/US]; 28 Radford Road, Princeton, MA 01541 (US). WISE, Donald, L. [US/US]; 195 Common Street, Belmont, MA 02178 (US). WNEK, Gary, E. [US/US]; 1103 Buckingham Station Drive, Midlothian, VA 23113 (US). LEWANDROWSKI, Kai-Uwe [DE/US]; 423 Washington Street #6, Brookline, MA 02146 (US).

(74) Agents: HEINE, Holliday, C. et al.; Weingarten, Schurgin, Gagnebin & Hayes LLP, Ten Post Office Square, Boston, MA 02109 (US).

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(54) Title: BIODEGRADABLE, BIOPOLYMERIC BIOELECTRET IMPLANT FOR TISSUE REGENERATION

(57) Abstract

Use of the electrical properties of biopolymers, in particular, α–helical polypeptides and the homo– and co–polymers formed from α–hydroxy acids, in materials for tissue growth and repair and other biological applications is disclosed. The invention features a biodegradable device for tissue growth and/or repair having at least one tissue contacting surface and comprising an electrically charged synthetic biodegradable, biopolymeric, bioelectret material characterized by a bulk monopolar charge that produces an external electrostatic field. Electrets are formed in the material of the device upon the application of an electrical voltage. The resultant biodegradable, bioelectret biopolymeric device is useful for promoting tissue growth (e.g., growth of nerve or bone tissue) and also as an aid in the final integration of a biodegradable device following transplantation.

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TITLE OF THE INVENTION

BIODEGRADABLE, BIOPOLYMERIC BIOELECTRET IMPLANT FOR TISSUE REGENERATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Patent Application Serial No. 60/061,526, filed October 9, 1997, the whole of which is hereby incorporated by reference herein.

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-- none --

BACKGROUND OF THE INVENTION

Although the production of viable tissues is the primary goal of tissue engineering, the integration of the engineered tissue into the host poses a serious challenge. The completion of this process is governed by two important processes, namely, innervation (i.e., formation of a neural network) and vascularization (i.e., formation of a blood vessel network). The innervation aspect for organ integration is known to require a lengthy period of time, up to 27 weeks in the case of intestinal transplantation.

The current clinical standard of care for peripheral nerve innervation or to repair peripheral nerve injury is to perform a peripheral nerve graft as a conduit through which nerve regeneration can occur. Although the technology of microsurgical repair is fairly routine, only approximately 50% of the regenerating axons are actually able to function across a gap through a nerve graft and successfully find the specific target end organ. In an attempt to improve upon these clinical results, synthetic guidance channels have been evaluated experimentally to repair severed peripheral nerves and offer promise for clinical nerve repair. The conduit allows the regenerating axon growth cone to find its appropriate target organ.

In recent years, investigators have evaluated a number of nerve conduits. Some of these are derived from biomaterials (i.e., nerve, muscle, vein or pseudo synovial sheath) or artificial tubes. The permanent non-resorbable artificial material has the potential disadvantage of either extrusion, infectious complications or compression and restriction of the peripheral nerve regeneration process due to confined space and the potential need for removal at a later stage.

BRIEF SUMMARY OF THE INVENTION

The invention is directed to using the electrical properties of biopolymers, in particular, α-helical polypeptides and the homo- and co-polymers formed from α-hydroxy acids, in materials for tissue growth and repair and other biological applications. The invention features a biodegradable device for tissue growth and/or repair having at least one tissue contacting surface and comprising an electrically charged synthetic biodegradable, biopolymeric, bioelectret material characterized by a bulk monopolar charge that produces an external electrostatic field. Electrets are formed in the material of the device upon the application of an electrical voltage. The resultant biodegradable, bioelectret biopolymeric device is useful for promoting tissue growth (e.g., growth of nerve or bone tissue) and also as an aid in the final integration of a biodegradable device following transplantation.

In one embodiment, the biodegradable device of the invention further comprises a biodegradable, biopolymeric scaffold and the electrically charged synthetic biodegradable, biopolymeric material is incorporated within said scaffold. Preferably, the bioelectret of the device is a ferroelectric biopolymer and most preferably an α-helical polypeptide, for example, a poly-L-glutamic acid alkyl ester such as poly(γ-methyl-L-glutamate).

Other biodegradable polymers that can be given bioelectret properties and, therefore, can be useful in the

device of the invention include polydioxanone, poly(ϵ-caprolactone); polyanhydride; poly(ortho ester); copoly(ether-ester); polyamide; polylactone; poly(propylene fumarate) ($H[-O-CH(CH_3)-CH_2-O-CO-CH=CH-CO-]_nOH$); and combinations thereof. Preferably, the polymer poly(lactide-co-glycolide) (PLGA: $H[-OCHR-CO-]_nOH$, R=H, CH_3), with a lactide to glycolide ratio in the range of 0:100% to 100:0% inclusive, is used. The above-mentioned biopolymers are also useful as scaffolding material in the device of the invention.

As many of the preferred biodegradable polymers from which the device of the invention is manufactured are polymers that can produce acidic products upon hydrolytic degradation, the device may also include a neutralization compound, or buffer. The neutralization compound can be included in sufficiently high concentration to decrease the rate of pH change as the device degrades, in order to prevent sterile abscess formation caused by the accumulation of unbuffered acidic products in the area of device placement.

In another alternative embodiment, the biodegradable device of the invention preferably includes a biological growth factor, e.g., a nerve or tissue growth factor, to enhance cell growth.

As used herein, the term "biodegradable" is defined as the biologic elimination of the products of degradation by metabolism and/or excretion and the term "bioerodible" is defined as the susceptibility of a biomaterial to degradation over time, usually months. An "electret" is defined as a dielectric body in which a permanent state of electric polarization has been established. Electret material is characterized by a bulk monopolar charge that produces an external electrostatic field. The term "ferroelectric" refers to a substance having remanent electric polarization that is reversible by an electric field. The terms "neutralization compound" or "buffer" are defined as any material that limits or moderates the rate

of change of the pH in the guide and its near environment upon exposure to acid or base. The term "acidic products" is defined herein as any product that generates an aqueous solution with a pH less than 7.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims, taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a scanning electron micrograph showing a cross-sectional surface of a nerve guide foam scaffolding of the invention;

Fig. 2 shows a biodegradable nerve guide of the invention *in situ*;

Fig. 3 is a graph of PMLG current density versus field strangth, including background current;

Fig. 4 is a graph of PMLG current density versus field strangth, after correcting for background current; and

Fig. 5 is a PMLG ferroelectric hysteresis curve.

DETAILED DESCRIPTION OF THE INVENTION

In the presence of electroactive materials such as polypyrrole, rat nerve cells have been shown to respond to electrical stimulus by producing neurites that are twice as long as neurites produced in the absence of an electrical stimulus (Langer et al., Proc. Nat'l Acad. Sci., USA 94:8948, 1997). Furthermore, studies with electrically induced, non-biodegradable synthetic polymers such as fluoropolymers indicate that enhanced cell differentiation is related to charge properties of the substrata and is not dependent on autocrine or cell-derived factors alone. In addition, negatively charged, non-biodegradable polymers such as fluoropolymers have been shown to induce cell ingrowth and promote adherence of artificial prostheses (Valentini, Robert F., U.S. Pat. No. 5,759,205, June 2, 1998, which is incorporated by reference herein).

We have now determined that biodegradable, biopolymeric materials can also be made to hold an electric charge (become electrets) and mediate the beneficial effects of enhancing tissue (e.g., nerve or bone tissue) growth and/or repair. Previous work in our laboratory led to the development of biodegradable, biopolymeric porous foam scaffolds, e.g., for nerve regeneration (U.S. Patent No. 5,456,917, which is incorporated by reference herein). We have now demonstrated that films of, e.g., biodegradable polyglutamic acid esters exhibit ferroelectric (FE) properties when aligned in an electric field. To promote the electrical properties of the biopolymeric porous scaffold, biodegradable ferroelectric fibers have been incorporated into a biodegradable foam nerve guide. This nerve guide device with its integrated ferroelectric elements will permit the development of a practical clinical treatment for the repair of peripheral nerve injuries and allow the development of nerve conduits which result in significantly improved peripheral nerve regeneration compared with standard microsurgical nerve grafting procedures currently employed.

Electrets are provided to the biomaterial using a corona discharge apparatus. Due to surface charge traps and low internal conductances, electrets permanently store positive or negative monopolar charges injected into the biomaterial during electrical charging procedure by using a coronal device. The trapped monopolar charges generate a measurable external field whose polarity and magnitude is related to the polarity and number of internal charges. Projected surface charge densities can be accurately quantified using non-contact voltage measurements techniques. It is expected that tissues grown on electrically charged biodegradable biopolymeric substrates will display greater levels of differentiation and faster outgrowth than tissues cultured on uncharged substrates.

Electroactive biopolymers such as the simple alkyl ester of polyglutamic acid, poly(γ-methyl-L-glutamate)

(PMLG), which are biodegradable, are excellent materials for use in the fabrication of interactive scaffolds for enhanced nerve regeneration when formed into fibers and embedded in a biodegradable foam guide. After assuring the outgrowth of the nerve fibers toward the distal nerve stump and maturation of the regenerated nerve fibers, the nerve guide of the invention gradually will degrade.

PMLG is a hydrogen bonded α-helix biopolymer which was rendered ferroelectric by poling under high electrical field strengths (150 Mvolts/cm). The ferroelectric biomaterial maintained a charge of 655 mCoulombs/m^2 (the so-called remanent polarization, P_r) after releasing the voltage. In comparison P_r is 126 mC/m^2 for nylon-5 and 52.6 mC/ m2 for polyvinylidene fluoride (PVDF) (Mei et al., Ferroelectrics 144:51-60, 1993). It is this property of charge retention that distinguishes these from other electroactive materials such as conductive polymers which require an external charge transfer (Cattaneo, M.V., "Ferroelectricity and Piezoelectric Biopolymers" in Electrical and Optical Polymer Systems: Fundamentals, Methods and Applications, Wise et al. eds, Marcel Dekker, Inc., NY, pp. 1213-1222, 1998).

The biopolymer PMLG, and other α-helix biopolymers, offer definite advantages compared to the non-biological PVDF as a substrate for neurite cell growth. First and foremost, PMLG as a biopolymer can ultimately be incorporated into any cell tissue outgrowth, e.g., for nerve or tissue repair, without requiring its withdrawal due to long-term tissue incompatibility or rejection. In addition, the higher ferroelectric properties of PMLG compared to PVDF translates into higher electrical activities to stimulate nerve outgrowth.

These results suggest that incorporation of PMLG into devices for tissue engineering as coatings or fibers should provide greater benefit than the use of fluoropolymers. PMLG-based electret materials can be fabricated into thin, transparent films. Among the biodegradable polymers the

homo- and copolymers derived from alpha-hydroxy acids, namely glycolic acid and lactic acid, represent the largest family of synthetic polymers currently in *in vivo* clinical use. The highly crystalline polylactic biopolymer (PLLA) has the highest piezoelectric constant among polymer crystals (Fukada, E., Plastics Eng. 28:393-434, 1995). This polymer can be made electroactive by forming electrets into the PLGA material using similar procedures as those applied to the fluoropolymers and the PMLG biopolymer (Fukada, E., IEEE Trans. Elect. Ins. 27: 813-819, 1992). The bioelectret form of polylactic acid (PLLA) could be used in the form of rods for intramedullary pinning and in the form of films for enhancing periosteal or dental growth.

We are also able to promote the electrical conductivity of PLGA nerve guides by adding carbon (graphite) and gold fibers as a core inside the lumen of the nerve guide.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

EXAMPLE 1

Preparation and testing of biodegradable, biopolymeric porous foam scaffolds for use in the nerve guide of the invention.

We have prepared polymeric foams of poly(lactide-co-glycolide) (PLGA) by lyophilization of solutions of the PGLA polymer, as described in U.S. Patent No. 5,456,917. The foams are open-celled materials which are ideal templates for cell growth. For cell growth requiring attachment, an open-celled porous scaffold prepared from a biodegradable (resorbable) polymer has the following advantages: (a) a large surface area is available for attachment, depending on pore size and foam density; (b)

the polymer degradation by hydrolysis allows gradual increase of contiguity as the polymer erodes; and (c) the open-celled structure permits the continuous exchange of nutrients for growth without cell damage, and provides a support for exogenously added biological growth facilitators. A nerve guide formed from the PLGA polymeric foam can easily be made electroactive as described above.

Our PLGA foams show characteristic densities dependent on the concentration of polymer in solution. As shown in Fig. 1, scanning electron micrographs (SEM's) of PLGA foams prepared as described show a pore size distribution (median 15-20) which depends on the location within the foam, with smaller pores near the surface. The surface of the foam, although still porous, is less so than the interior. This overall architecture is typical for all densities and offers high porosity for regenerative areas and low porosity for areas of foreign cell invasion. The biopolymeric foam scaffold was tested, as described below, for support of Schwann cell attachment and vascular neogenesis and the ability to support nerve regeneration across gaps of 10 mm.

Ten mm segments of sciatic nerves were resected from outbred Sprague-Dawley rats. An 85:15 PLGA polymer foam guide, sterilized by γ-irradiation, was then placed to span these 10 mm gaps. Nerve regeneration was assessed at four weeks by axon counting, EMG, transmission and scanning electron microscopy. Nerve regeneration across 10 mm gaps through standard polyethylene guides served as controls.

At four weeks, regenerated nerve cables were present across the entire 10 mm gap in all experimental animals (n=4). Axon counts were significantly elevated in the experimental group compared with controls (4616 $\pm$ 475 versus 1850 + 897 ($x \pm$ SEM), p = 0.017). Nerve conduction velocity was also superior: 12.27 $\pm$ 4.21 m/s versus 4.3 + 1.34, p = 0.044. As can be seen in Fig. 2, marked vascularity was noted throughout the regenerated nerve cables only in the polymer scaffold guides.

Identification of the ferroelectric properties of α-helical biopolymers.

Esters of poly-L-glutamic acids (PRLG's), such as the benzyl and the methyl esters, PBLG and PMLG, respectively, form α-helical polymers when cast from appropriate solvents and are known to display piezoelectric (PE) properties when aligned in magnetic or electric fields. Analysis of the molecular conformation of the aligned dipoles suggests that the direction of the dipole vector in aligned films may be reversed by application of an electric field, thus giving rise to ferroelectric (FE) properties. The thermal stability of some PRLG's, their facile film forming capacity, and the ease of dipole alignment make these materials useful in applications exploiting PE and FE behavior in transducers and electro-optical switching.

We have determined that films of polyglutamic acid esters, well known as piezoelectric polymers, also demonstrate ferroelectric (FE) properties when aligned in an electric field. When poled, PMLG showed a pronounced FE response characterized by a remanent polarization, Pr, of 655 mC/m^2 and a coercive field strength, Ec, of 120 MV/m. The Pr value is decidedly higher than observed for other FE polymers. For nylon-5 the Pr value is 126 mC/m^2 (Ec = 100 MV/m) and for poly(vinylidene fluoride), PVF_2, Pr = 52.6 mC/m2 (Ec = 50 MV/m).

Structural advantages accrue from use of FE polymers: polymers may be fabricated as thin films or fibers with flexibility and strength, and a variety of forms are possible. The ease with which PRLG's, such as PMLG, can be ordered in an externally applied electric field to create a film or fiber with strong dipole alignment and exhibit ferroelectric properties means that other self-organizing and alignable polymers will be suitable for development of FE materials.

Poly(γ-methyl-L-glutamate), PMLG, is a synthetic polypeptide which is known to assume α-helical conformation

in many solvents. Typical helicogenic solvents include partially halogenated hydrocarbons such as dichloromethane, and dichloroethylene, as well as dioxane and m-cresol (Block, 1983). The helical coil is stabilized by hydrogen bonds directed from the amide nitrogen proton of a residue to the carbonyl oxygen of a residue four units behind it. The individual dipoles arising from these hydrogen bonds are parallel to the helical axis and, having the same direction, give rise to a large cumulative molecular dipole which is molecular weight dependent. Residue dipole moments have been summarized by Block. These range from 0.6 to 1.37×10^{-29} coulomb-meters (1.8-4.1Debye).

In a demonstration of the ferroelectric effect, PMLG was dissolved in m-cresol at 100°C to form a 2% by weight solution. The solution was cast on clean glass slides, which were then dried in vacuum at 95°C for three hours. The films were removed from the slide by immersion in deionized water and again dried. From the volume of solution used and its concentration, the polymer content of the films was calculated to be 85%, indicating the presence of about 15% residual cresol which acted as a plasticizer. The thickness of the films was approximately 15 μm. Gold electrodes were evaporated on each side of the film; the overlapped electrode area was 0.5 cm by 1.4 cm. The films were poled in dry silicone oil bath at room temperature. A field sweeping technique was employed to minimize ionic conduction. The field was incrementally increased to 150 MV/m overnight before poling. After the field sweeping procedure, the sample was then poled at a maximum applied field of ±150 MV/m. The applied electric field and current were recorded, and from these data the current density versus electric field strength was plotted. Fig. 3 shows the plot including background current. Fig. 4 shows the same data after correcting for background. Integration of these data gives the electric displacement versus electric field curve, that is, the ferroelectric hysteresis loop shown in Fig. 5. The remanent polarization for PMLG is 655

mC/m^2. In comparison Pr is 126 mC/m^2 for nylon-5 and 52.6 mC/m^2 for PVF2. The coercive field strengths for these three polymers are respectively 120, 100, and 50 MV/m (Mei et al., 1993).

The ferroelectric response can be measured on films or fibers that have been prepared in the absence of an electric field and also on similar films which have been prepared by solvent evaporation in the presence of an aligning field. The field is applied parallel to the plane of the solution in an electrode cell machined from a teflon block.

To prepare a film for piezoelectric or ferroelectric measurements, electrodes, e.g., aluminum or gold, are positioned on both sides of the film with an offset of 3 mm to facilitate cementing of the leads. Aluminum electrodes, made from 13 micron foil (Fisher Scientific), are cemented to the film with Silver Print Conductive Cement from GC Electronics (Rockford, IL). Gold electrodes are vapor deposited to a thickness of 250 Angstroms using Edwards Coating System E306A. Gold wire, the electrode source material, is obtained from Ernest F. Fullam, Inc. (Latham, NY, Cat. No. 12201). Prior to fixing the electrodes in place, film thickness is measured with a Starrett Electronic gauge (The L.S. Starrett Co., Athol, MA, Cat. No. 812-14).

Piezoelectric measurements are made on a Toyoseiko Piezotron system comprising the thermocontrol, interface and analog units. Measurements will yield values for the dielectric constant, the piezoelectric d- and e- constants, and Young's modulus. Following these measurements, the ferroelectric hysteresis can be examined. Films are immersed in dry silicone oil (Dow Corning), through which a current of dry air is passed to maintain anhydrous conditions. Leads from the film are connected to a Trek Model 620A (±20 kV) amplifier. The electric field is scanned with a Keithley 617 Programmable Electrometer and

the current density monitored with a Keithley 195A Digital Multimeter.

In order to reduce ionic conductivity, and thus dielectric breakdown, samples are pretreated before poling by application of a static electric field. The purpose of this technique ("sweeping") is to remove the most mobile ionic impurities. Sweeping has been shown to reduce dramatically the ionic conduction during the following poling sequence (Mei et al., supra).

Other structural advantages also accrue from use of ferroelectric polymer films, e.g., strength, flexibility, light weight, and fabrication in many forms. Therefore, ferroelectrics find many additional applications, such as in pyroelectric detectors, ultrasonic and electroacoustic transducers, and ultrasonic light modulators.

Preparation and testing of a ferroelectric biopolymer nerve guide.

Having proved the validity of ferroelectric behavior in PMLG, and the potential for nerve regeneration in a biopolymeric guide made of PLGA foam, we have developed a PLGA foam guide containing PMLG fibers.

Polymer Selection and Characterization: Biodegradable polymers that are useful in the biopolymeric nerve guide of the invention, particularly as scaffolding material, include polydioxanone, poly(ε-caprolactone); polyanhydride; poly(ortho ester); copoly(ether-ester); polyamide; polylactone; poly(propylene fumarate) ($H[-O-CH(CH_3)-CH_2-O-CO-CH=CH-CO-]_nOH$); poly(lactic acid); poly(glycolyic acid); poly(lactide-co-glycolide); and combinations thereof. Selection of a particular polymer is based primarily on the known properties of the polymer, such as the potentiality for cross-linking, polymer strength and moduli, rate of hydrolytic degradation, etc. One of ordinary skill in the art may take these and/or other properties into account in selecting a particular polymer for a particular

application. Thus, the selection of a particular polymer is within the skills of the ordinary skilled practitioner.

In a preferred embodiment, the polymer poly(lactide-co-glycolide) ($H[-OCHR-CO-]_nOH$, R=H, CH_3) (PLGA) is used. The PLGA polymers used according to the invention desirably have a lactide to glycolide ratio in the range of 0:100% to 100:0%, inclusive, i.e., the PLGA polymer can consist of 100% L- or D,L-lactide (PLA), 100% glycolide (PGA), or any combination of lactide and glycolide residues. These polymers have the property of degrading hydrolytically *in vivo* to form organic acids (lactic acid and glycolic acid) which accumulate in the region surrounding the guide. These acids are metabolized and eventually excreted as carbon dioxide and water or enter the citric acid cycle.

A particularly preferred polymer for use in the nerve guide of the invention is poly(d,l-lactide-co-glycolide)-85:15 (Boehringer-Ingelheim: distributor, Henley Chemicals, Inc., Montvale, NJ), the 85:15 designation referring to the lactide to glycolide mole ratio. The particularly preferred polymer is Resomer™ RG 858, with an inherent viscosity of approximately 1.4 corresponding to a weight average molecular weight of 232,000 as measured by gel permeation chromatography (GPC).

The polymer can be used as received or purified by precipitation from tetrahydrofuran solution into isopropanol, air dried and then exhaustively vacuum dried. Polymer data (composition and molecular weight) can be confirmed by nuclear magnetic resonance and by GPC (Hsu et al., J. Biomed. Mater. Res. 35:107-116, 1997).

Bioelectric fiber preparation: Alignment and Polarization: Ferroelectric PMLG fibers are produced using an hydraulic press (Model 10060 Enerpac, 100 tons capacity, H frame). A corona poling device is used to render the extruded PMLG fiber ferroelectric. A voltage d.c. field up to 150 MV/cm is applied using a low current, d.c. power supply with reversible polarity outputs (Bertan Associates, Inc. model 205-50R, Syosset, NY) across the thickness of

the fiber to align dipoles and form electrets located in the polymer crystals. PMLG discs, e.g., 5 cm^2, can also be charge injected (fabricated into bioelectrets) using a corona-charging apparatus.

Due to subsurface charge traps and low internal conductances, electrets permanently store positive and negative monopolar charges injected using the corona discharge apparatus. The trapped monopolar charges generate a measurable external field whose polarity and magnitude is related to the polarity and number of internal charges. Projected surface charge densities can be accurately quantified using non-contact voltage measurement techniques.

Ferroelectric PLGA foam nerve guides: Ferroelectric PLGA foam guides are prepared by lyophilization (freeze drying) of the PLGA solutions in a mold containing the PMLG fibers and preformed to give cylindrical implants with a 4.0 mm O.D. and a 2.0 mm I.D. The density of the foam laminate and its void volume are a function of the concentration of the polymer in the solution. A low density (0.1 g/cm^3) foam can be prepared starting with 100 mg/ml polymer solution. Lyophilization at dry ice temperatures (-78 °C) yields a polymer foam with a median pore size of 15-20 μm. Foam structure is confirmed by scanning electron microscopy (SEM). Void volume and pore size distribution is determined by mercury intrusion porosimetry (MIP). Density is measured by standard gravimetric techniques as applied to solids. Together, density and solid volume measurements enable calculations of the ratio of open to closed cells (Hsu et al., supra). The electric field is scanned with a Keithley 617 Programmable Electrometer and the current density monitored with a Keithley 195A Digital Multimeter.

In Vivo Evaluation of Nerve Regeneration: Following peripheral nerve injury, the neuronal cell body undergoes reactive changes aimed at restoring functional contact with its end-organ. These changes in the perikaryon promote

axonal out growth at the injury site and, if the distal environment is favorable, successful regeneration through contact with a target. The distal nerve segment is a portion of the neuronal population, either in the dorsal root ganglia or in the ventral horn of the spinal cord, undergoes cell death. The beneficial effects of nerve growth factor in the presence the neuronal death that normally occurs in the dorsal root ganglion, ventral horn after axotomy and in the enhancement of peripheral nerve regeneration have been reported.

In vivo evaluation is carried out in rats. The foam matrix alone (Group 1), the foam matrix plus PMLG fibers (Group 2), and the experimental control of standard nerve grafts (Group 3). In the study procedure, the right sciatic nerve is exposed through a gluteal muscle splitting incision and transected at the mid thigh level using straight micro dissecting scissors. In all groups two operations are performed. In the first operation the rat's sciatic nerve are transected and a 10 mm segment removed. The incision is closed and the animal permitted to recover. The resorbable foam guide is ultimately to be placed back into the same animal from which the Schwann cells were derived. This process should take approximately one week.

One week later, in all groups, the animals undergo a second operation with exposure of the right sciatic nerve, resection of a traumatic neuroma, and implantation of the polymer guides in the 10 mm gap with two 10-0 nylon sutures, inserting the proximal and distal nerve stumps 2 millimeters into the polymer guide (4.0 mm OD, 2.0 mm ID, and 14 mm long). The muscle is closed with 3-0 chromic sutures and the skin is closed in 4-0 Dexon subcuticular suture. Peripheral nerve regeneration is assessed in 4 ways: histologically, electrically, morphologically, and functionally (Bryan et al., J. Reconstr. Microsurg. 12:439-446, 1996). Analysis of data from these four techniques will allow one to assess the degree of peripheral nerve regeneration compared to the experimental control of a

nerve graft. The histological data will allow assessment of the number of myelinated and non-myelinated regenerated axons, the thickness of the myelin sheath around the myelin axons, the degree of revascularization of the regenerated nerve cable, and the presence or absence of collagen scar.

Electrophysiologic studies determine whether or not the histologically observed regenerated axons have functional capability in terms of generating an action potential providing reinnervation of the motor end plate of the target muscle. Lastly, tracking studies provide a functional assessment of the peripheral nerve that is regenerated in not only confirming histologically and electrically that an axon has regenerated across the entire gap through the matrix guide, but that an appropriate target end organ has been reinnervated which allows the animal to contract the muscle and have gait analysis performed. Each of these parameters are compared to the gold standard of the nerve gap spanned by nerve graft.

For the appropriate histologic studies, six animals from each group are sacrificed three days, seven days, one month, and three months after the second operation. Axon counts and myelineation index are calculated. The regenerated cable is removed from the polymer guide, fixed in 2.5% glutaraldehyde and 2% cerium, and embedded in epon. Thin sections are cut, mounted on slides, and stained with toluidine blue dye. Axon counts are performed on a Nikon Optiphot microscope interfaced to a Panasonic Tr-124 MA video monitor, image processor, and an IBM APC. An approximate distinction is made between myelineated and unmyelinated axons under light microscopy. The myelination index (ratio of myelin to axonal area) is also calculated. The gross axon counts confirm histologically peripheral nerve regeneration and will confirm that the regenerated nerves are myelineated to a comparable degree to that which is normally measured. These data also allow internal correlation between EMG data and the maturity of the

regenerated axons since there is a direct correlation of neurovascularization of the regenerated nerves.

Electromyography is performed on all animals before sacrifice. The sciatic nerve is exposed at the sciatic notch proximal to the repair conduit. A recording needle is placed in the gastrocnemius muscle 10 mm below the tibial tubercle. The nerve is stimulated supramaximally with two silver wire electrodes. Compound action potentials are measured, and the earliest latency and peak amplitudes were recorded. The distance from the sciatic notch to the gastrocnemius muscle is found through dissection (generally, a mean length of 50 mm). The EMG studies confirm regeneration of the peripheral serve across the entire 10 mm gap and reinnervation of the motor end plate of the muscle.

For morphological characterization, electron microscopy is performed to evaluate the ultra structure of the regenerated nerves and the relationship of the nerves to the biodegradable polymer. High power SEM shows ingrowth of axons and vessels. TEM of cross section from experimental groups shows axons and polymer.

Functional assay is performed by walking track analysis. Animals are sacrificed at 4 and 12 weeks before induction of anesthesia, tracking studies are performed by painting the hind feet with methylene blue dye and having the animals walk. The sciatic function index (SFI) is calculated by comparing prints with those taken before surgery.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A biodegradable device having at least one tissue contacting surface, said device comprising an electrically charged synthetic biodegradable, biopolymeric, bioelectret material, said material characterized by a bulk monopolar charge that produces an external electrostatic field.

2. The device of claim 1, wherein said electrically charged synthetic biodegradable, biopolymeric material is ferroelectric.

3. The device of claim 1, further comprising a biodegradable, biopolymeric scaffold, wherein said electrically charged synthetic biodegradable, biopolymeric material is incorporated within said scaffold.

4. The device of claim 3, wherein said scaffold is porous.

5. The device of claim 1, wherein said electrically charged synthetic biodegradable, biopolymeric material is poly(lactide-co-glycolide).

6. The device of claim 3, wherein said scaffold comprises poly(lactide-co-glycolide).

7. The device of claim 1, wherein said electrically charged synthetic biodegradable, biopolymeric material is an α-helical biopolymer.

8. The device of claim 7, wherein said α-helical biopolymer is a polypeptide.

9. The device of claim 7, wherein said α-helical biopolymer is a poly-L-glutamic acid alkyl ester.

10. The device of claim 1, wherein said device is formed for use as a nerve guide.

11. The device of claim 1, wherein said device is formed for use as a coating for a tooth.

12. The device of claim 1, wherein said device is formed for use as a periodontal, odontal or oral maxillofacial graft, implant, cement or scaffold.

13. The device of claim 1, wherein said device is formed for use as an orthopaedic graft, implant, cement or scaffold.

14. The device of claim 1, wherein said device is formed for use as a neurosurgical graft, implant, cement or scaffold.

15. The device of claim 1, further comprising a buffering or neutralization compound in sufficiently high concentration to decrease the rate of pH change as said device degrades.

16. The device of claim 1, further comprising a biological growth factor.

17. The device of claim 16, wherein said biological growth factor is a nerve or tissue growth factor.

18. The device of claim 1, further comprising a core structural portion, wherein said electrically charged synthetic biodegradable, biopolymeric material forms a coating on said core structural portion.

19. The device of claim 18, wherein said core structural portion comprises carbon, ceramic or metal fibers.

20. A biodegradable nerve guide comprising an electrically charged synthetic biodegradable, biopolymeric, bioelectret material, said material characterized by a bulk monopolar charge that produces an external electrostatic field.

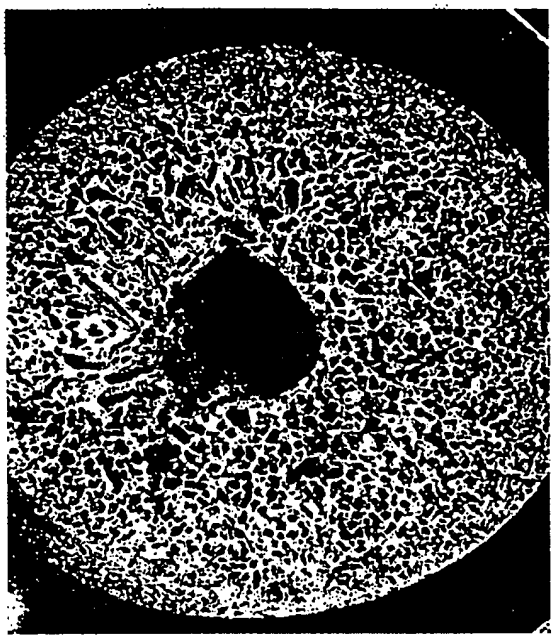

Fig. 1

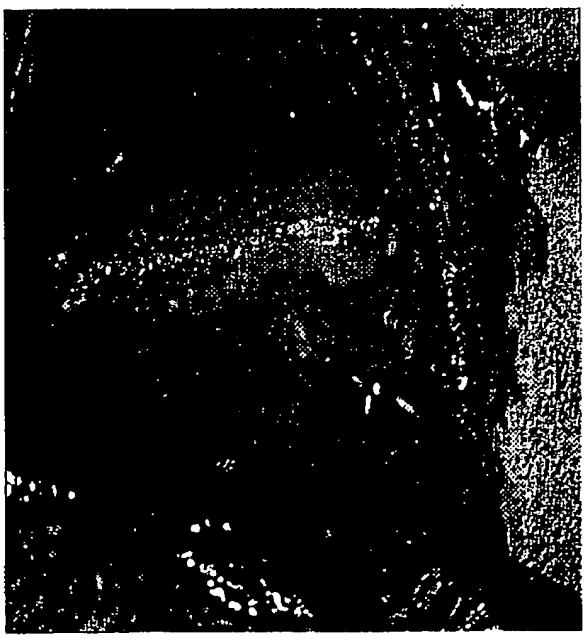

Fig. 2

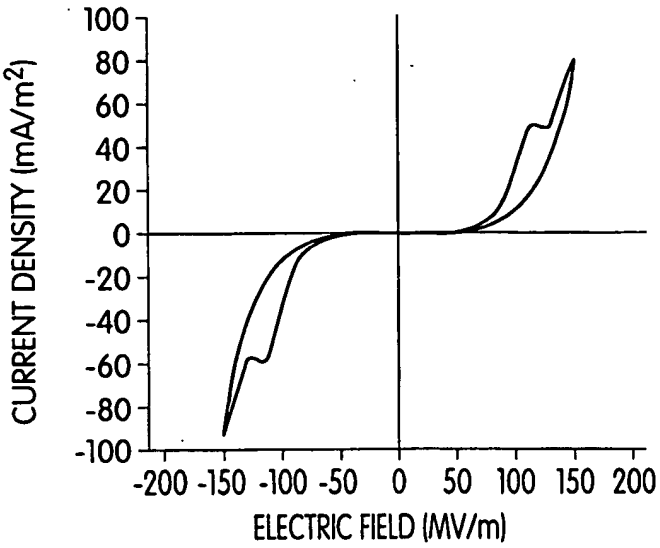

Fig. 3

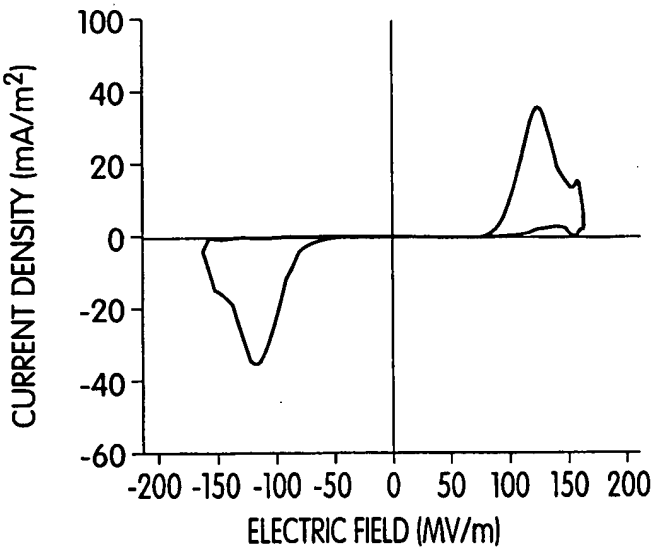

Fig. 4

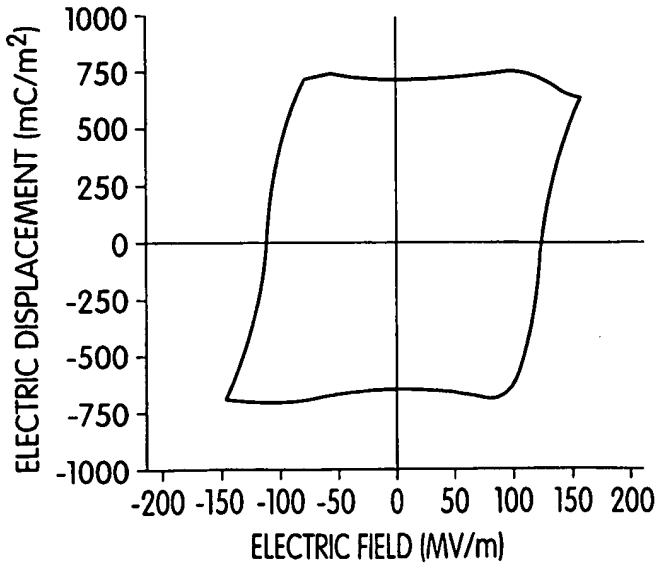

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21242

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61F 2/28; A61K 47/30
US CL :623/11, 16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 623/11, 16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 5,759,205 A (VALENTINI) 02 June 1998, entire publication.	1-20
Y	US 5,456,917 A (WISE et al) 10 October 1995, entire document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 JANUARY 1999

Date of mailing of the international search report

15 JAN 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer

Patricia Mark

DAVID J ISABELLA

Telephone No. (703) 308-3060